Hrvatska zaklada za znanost

Podrijetlo, udomaćenje i širenje graha (*Phaseolus vulgaris* L.)

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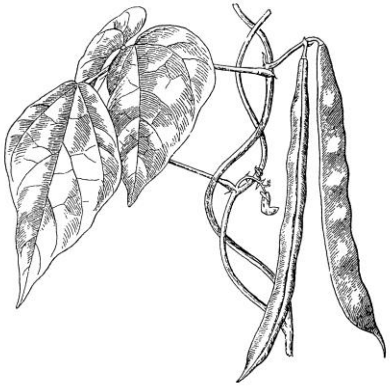
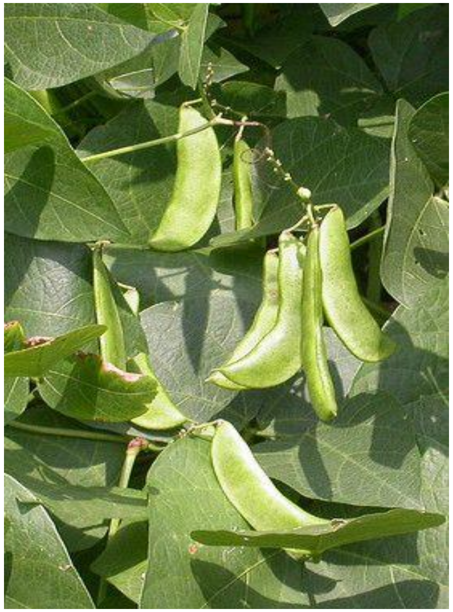


Zagreb, 11. lipnja 2015.

- rod *Phaseolus* sadržava 70-tak vrsta podrijetlom iz Centralne i Sjeverne Amerike
- pet vrsta je udomaćeno:

P. vulgaris

P. acutifolius

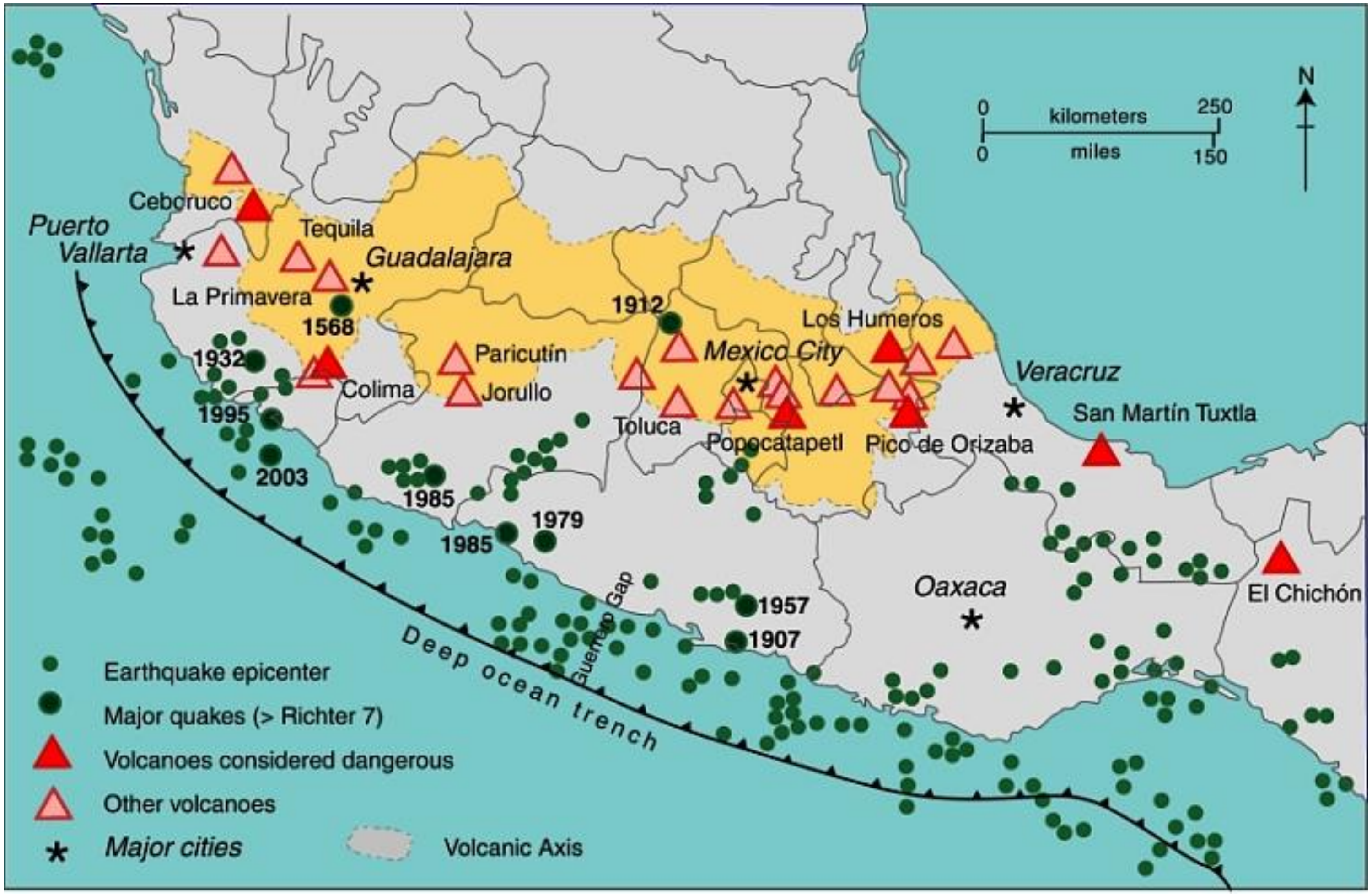


P. dumosus

P. lunatus

P. coccineus

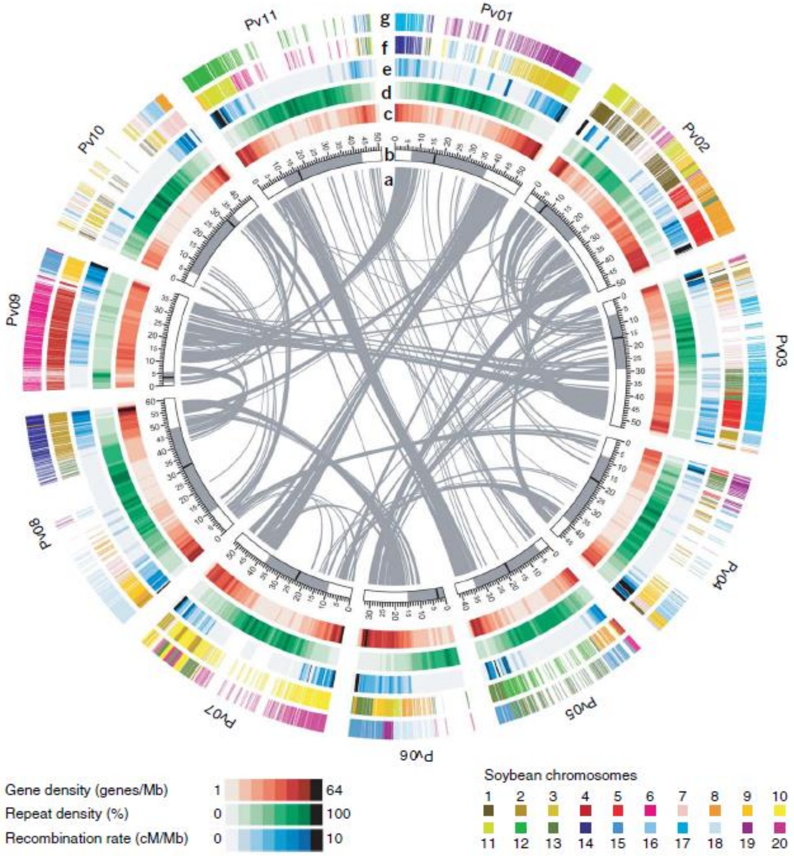
- sekvenciranje unutarnje transkribirajuće razmaknice ribosomske DNA (ITS) i kloroplastnog *trnK* lokusa (*Delgado-Salinas et al. 2006*) su omogućili datiranje roda na *ca.* 4–6 milijuna godina.



- obični grah (Phaseolus vulgaris L.) (2n =22)

~ 600 Mb, 27197 proteinski gen (Schmutz i sur. 2014)

- 91% proteinskih gena dijeli sa sojom



Struktura genoma graha i sintenija s genomom soje (*Schmutz i sur 2014*)

- za efikasnu zaštitu i upotrebu germplazme u razvoju novih poboljšanih kultivara izuzetno je važno istraživanje podrijetla i evolucije vrste

- divlji grah potječe iz Srednje Amerike (*Bittochi i sur. 2012, Schmutz i sur. 2014)*

- rasprostranjenje od Meksika do Argentine s dva eko-geografska genska skupa (djelomično reproduktivno izolirani)

- za andske divlje populacije je zabilježen višestruki prolazak kroz genetičko usko grlo (*Kwak and Gepts 2009, Rossi i sur. 2009*)

- udomaćenje se odvijalo istovremeno na dvije lokacije što je jedinstven scenarij kod kultiviranih biljaka (*Gaut 2014*)

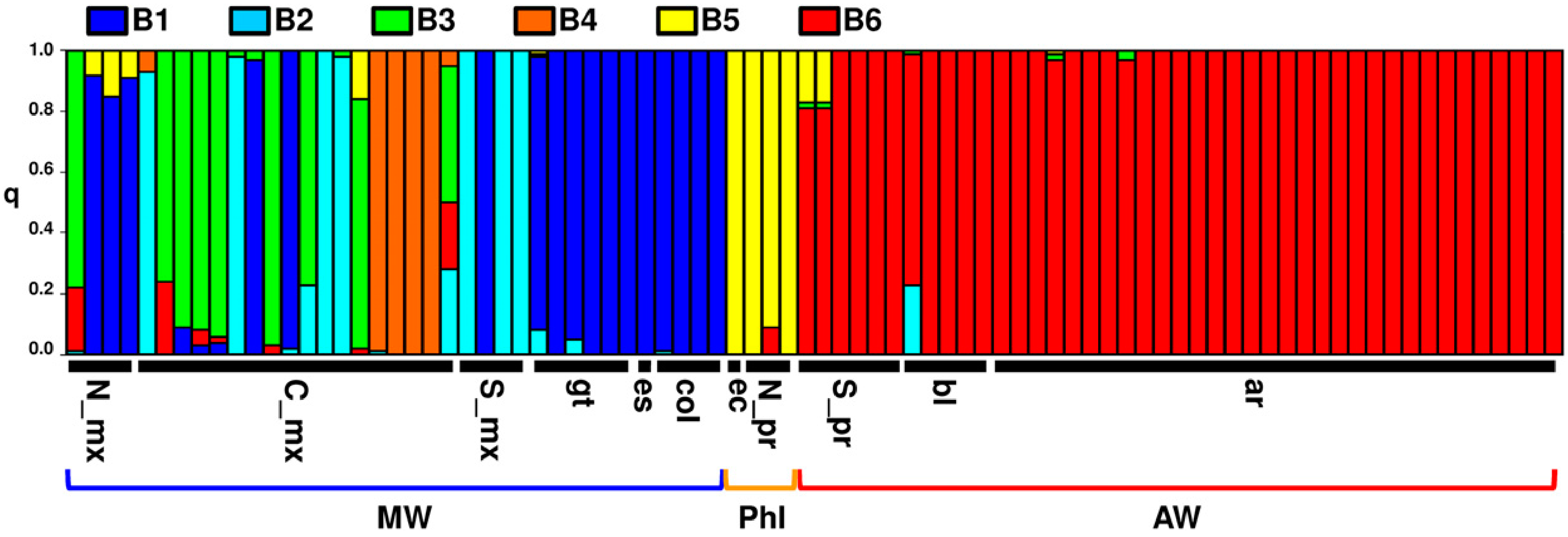
Milpa cropping system

- udomaćenje je dovelo do morfoloških promjena osobito u povećanju lista, sjemenki, boji i uzorku sjemenki, prilgodbama u odnosu na promjene u fotoperiodu i sl.

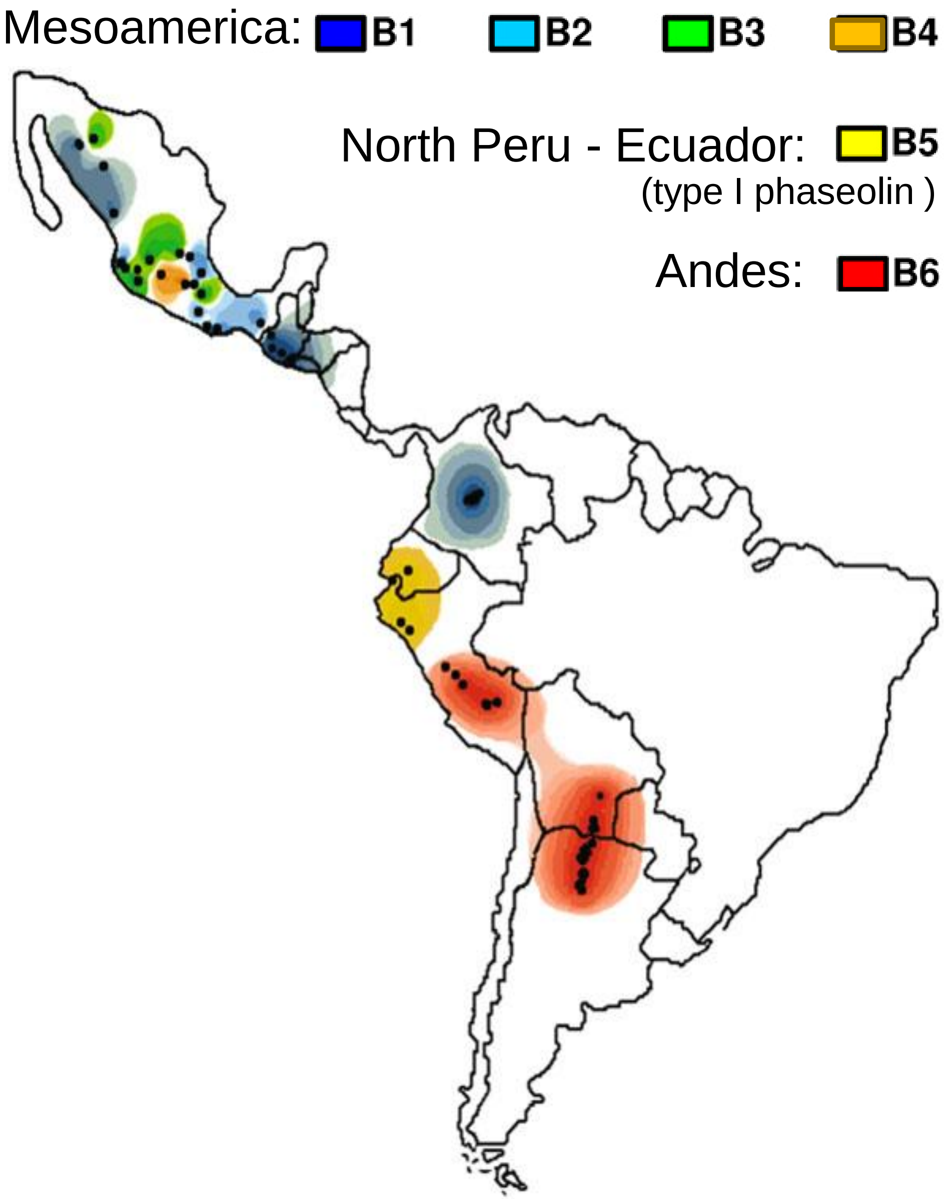
- identificirano je 1835 gena kod srednjeameričkog genskog skupa i 748 gena kod andskog genskog skupa povezanih s udomaćivanjem (manje od 10% zajedničkih) (Schmutz i sur. 2014)

- raznolikost sekvenci pet različitih gena u divljim populacijama (*Bittochi i sur. 2012; 2013):*

  leg044 - histidinol hidrogenaza
  leg100 – biotin sintetaza
  leg133 - dolicil-difosfooligosaharid protein glikoltransferaze,
  leg 223 - translacijski inicijacijski faktor SUI1
  PvSHP1 - kontrola otvaranja ploda



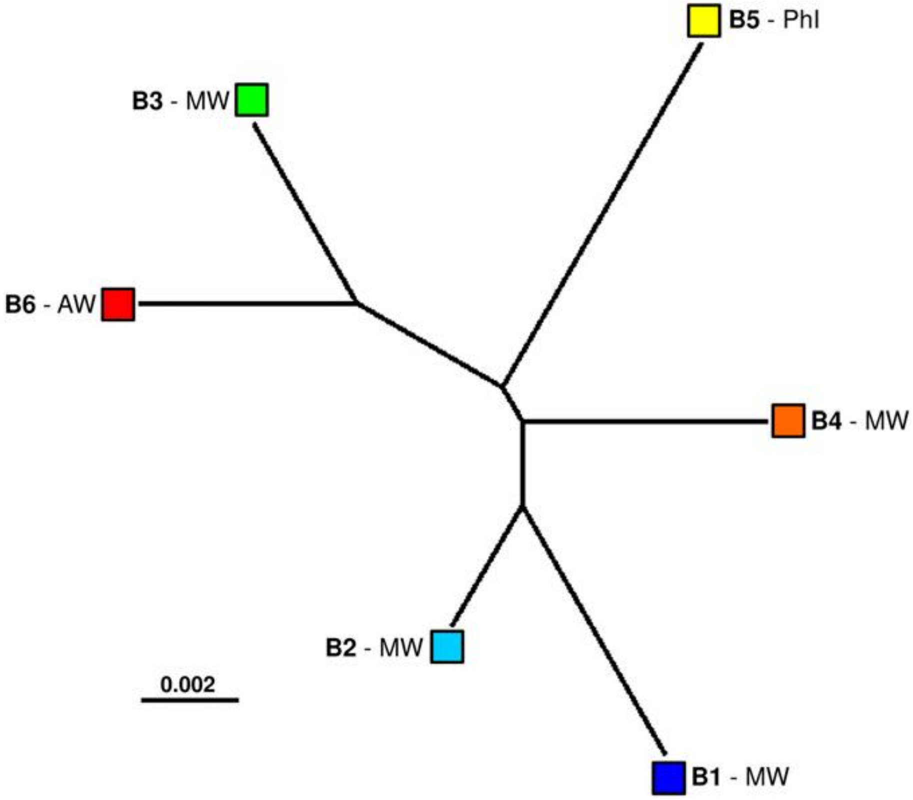
Populacijsko genetička struktura (Bayesovski pristup, BAPS 5.3.) (AW, Andean wild; ar, Argentina; bl, Bolivia; C_mx, central Mexico; col, Colombia; ec, Ecuador; es, El Salvador; gt, Guatemala; MW, Mesoamerican wild; N_mx, north Mexico; N_pr, northern Peru; PhI, type I phaseolin (northern Peru–Ecuador); S_mx, south Mexico; S_pr, southern Peru).



Mesoamerica: 🟦 **B1** 🟦 **B2** 🟩 **B3** 🟧 **B4**

North Peru - Ecuador: 🟨 **B5**
(type I phaseolin)

Andes: 🟥 **B6**

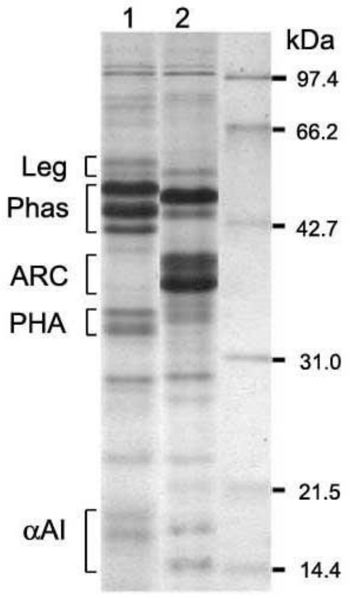


Nezakorjenjeno srodstvenao NJ stablo
temeljeno na raznolikosti sekvenca pet jezgrenih lokusa

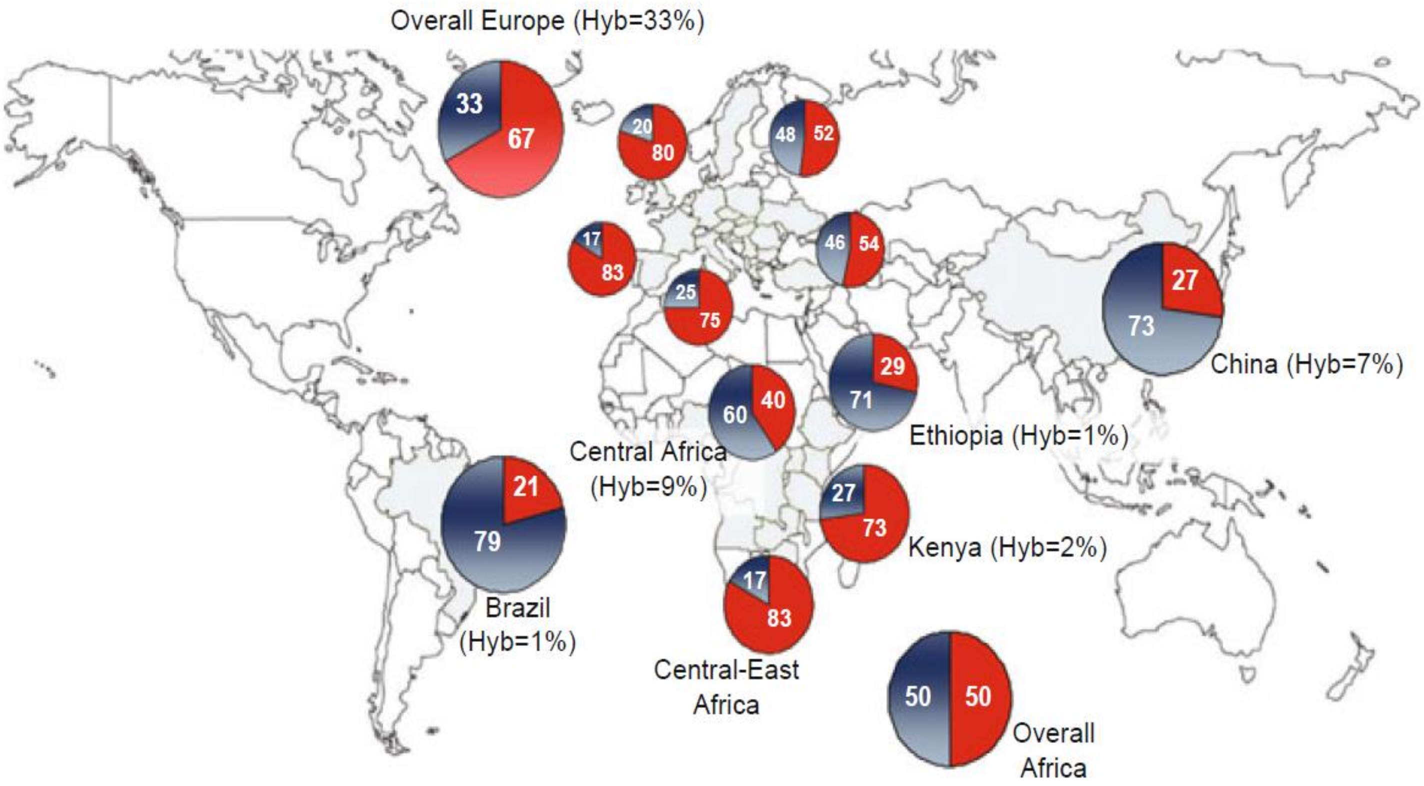
- putovi širenja graha izvan centara udomaćenja su vrlo kompleksni

- fazeolin je glavni spremišni protein u sjemenkama graha kodiran malom porodicom blisko smještenih gena

- prve studije su upotrebljavale izolaciju fazeolina iz sjemenki i SDS-PAGE elektroforezu kako bi razdvojili fazeolinske podjedinice i po njima razlikovali srednjeameričko od andskog podrijetla



SDS-PAGE: 1. cultivated bean cv. Tendergreen
2. wild bean, CIAT G12949



🟥 andsko podrijetlo ▨ srednjeameričko podrijetlo

prema Bellucci i sur. 2014

- konstruirane su PCR početnice unutar genske porodice za fazeolin koje umnožavaju mjesta s 15 i 21-bp duplikacijama specifičnih kombinacija ovisno o geografskom podrijetlu (srednjeameričkom /S tipovi/ ili andskom /T tipovi/) (Kami 1995)

- u sjevernom Peru i Ekvadoru su pronađene populacije divljeg graha kojima potpuno nedostaju duplicirane 15 i 21-bp regije (evolucijski najstariji tip fazeolina, I ili Inka tip)

```
  Exon 3 ↦ Intron 3
                                      21 bp repeat
I   TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGT...........
T1  TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGT...........
S1  TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGT...........
T2  TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGT...........
S2  TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGTCATCTAACTAC
T3  TTCAATGTAAGAAAGAAAACAGCATCTAACTACATATTTGCGTCATCTAACTAC

I   ..........TGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT
T1  ..........TGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT
S1  ..........TGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT
T2  ..........TGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT
S2  ATATTTTCGTTGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT
T3  ATATTTTCGTTGCCATTTAGCTAGTACTTTGTCTAAATGTCACACTTGTTGAAT

      *                                         ↦ Exon 4
I   TTGTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
T1  TTGTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
S1  TTGTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
T2  TTTTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
S2  TTGTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
T3  TTGTTGAATGATATCATTATATATGTTTGCATGATTTTTATAGAGCAAATTCGA
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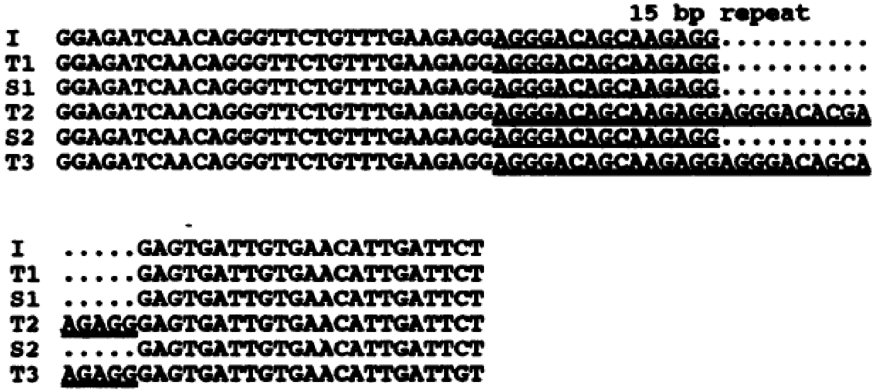
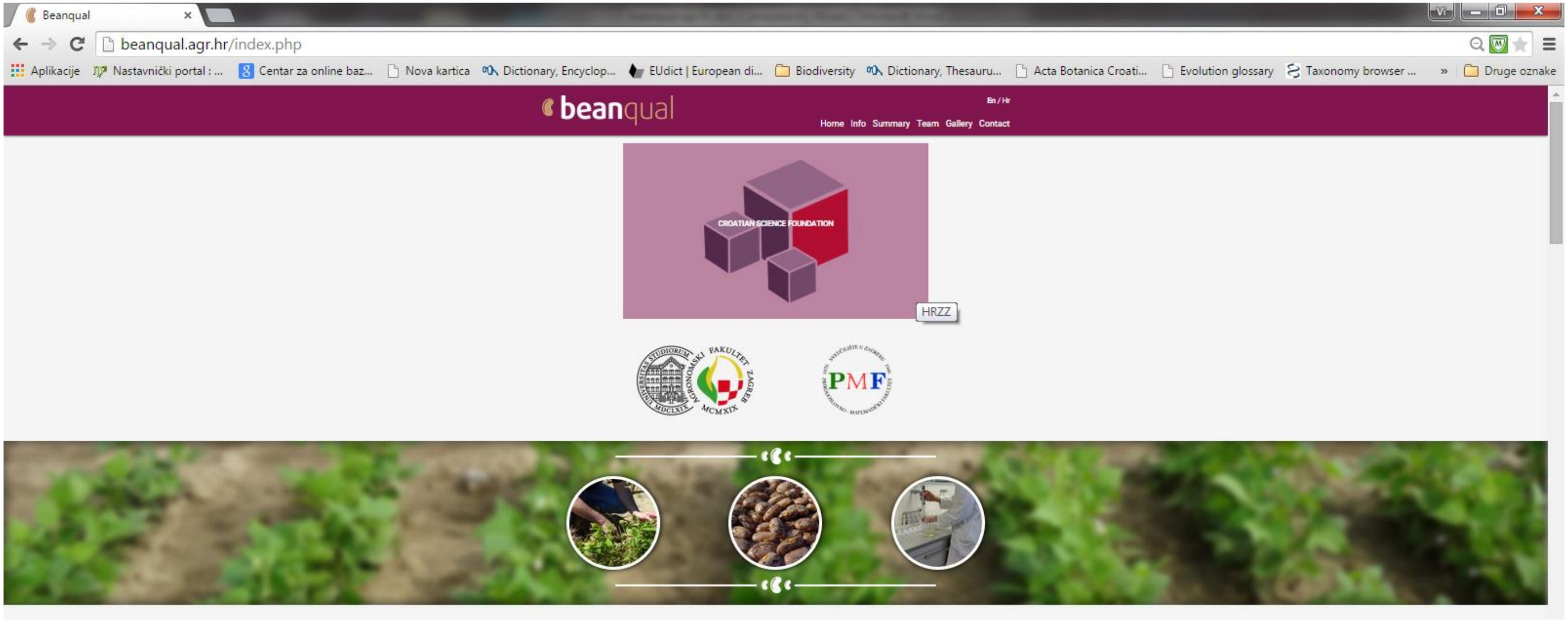


```
                                               15 bp repeat
I   GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGG..........
T1  GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGG..........
S1  GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGG..........
T2  GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGGAGGGACACGA
S2  GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGG..........
T3  GGAGATCAACAGGGTTCTGTTTGAAGAGGAGGGACAGCAAGAGGAGGGACAGCA

I   .....GAGTGATTGTGAACATTGATTCT
T1  .....GAGTGATTGTGAACATTGATTCT
S1  .....GAGTGATTGTGAACATTGATTCT
T2  AGAGGGAGTGATTGTGAACATTGATTCT
S2  .....GAGTGATTGTGAACATTGATTCT
T3  AGAGGGAGTGATTGTGAACATTGATTGT
```

Phaseolin type	Fragment size (bp)				Origin
I tip	268	/	/	/	PhI
S tip	268	/	289	/	MA
T tip	268	283	/	304	AN



Genetic Basis of Bioactive Nutritient Content in Croatian Common Bean Landraces

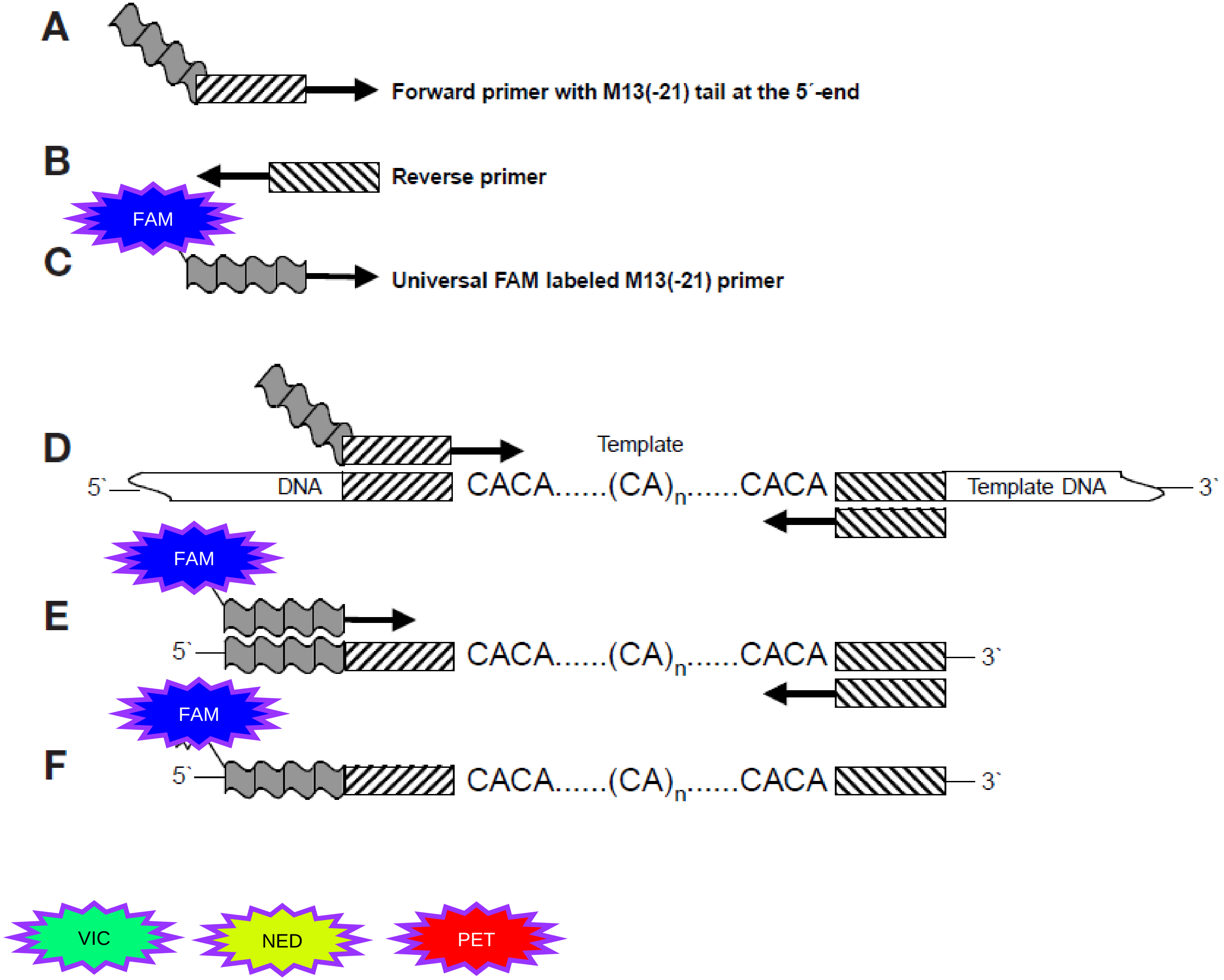
(1st of May 2014 - 30th of April 2017)

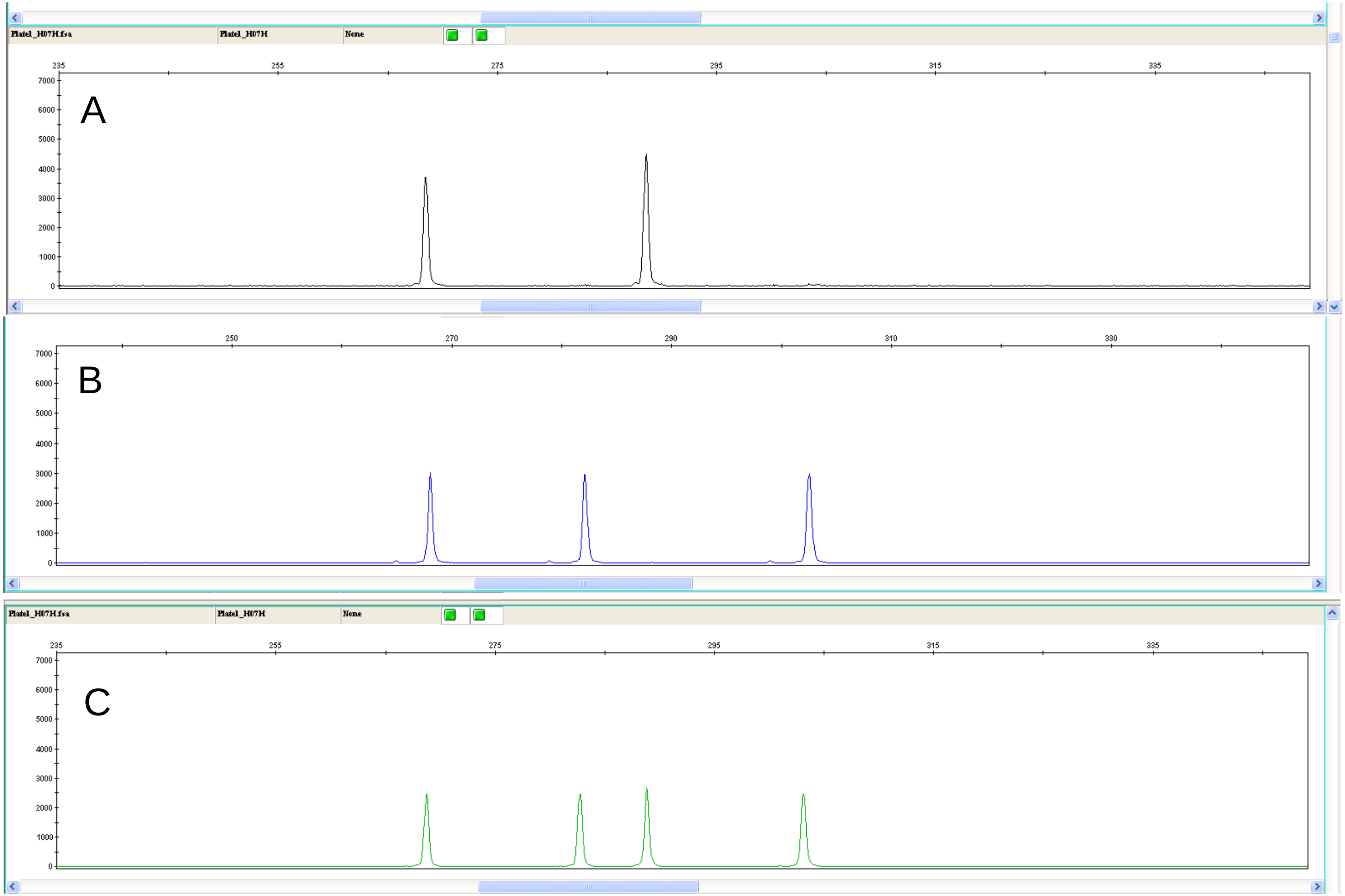
Doc. dr. sc. Klaudija Carović-Stanko
Svaučilište u Zagrebu, Agronomski fakultet

Objectives	Activities	Outputs – Milestones (M) and/or Deliverables (D)	Team members	Duration of activity (from-to, in months)
Multiplication and sampling	300 Croatian common bean landraces will be multiplied, leaf and seed samples sampled	Obtained material for analyses (D)	Klaudija Carović-Stanko Martina Grdiša Marko Petek Igor Palčić	from 2nd to 6th month
Yield potential assessment	Photosynthetic rates measurements	The assessment of diversity of 300 landraces (D)	Boris Lazarević Tomislav Karažija	from 3rd to 5th month
The assessment of diversity in seed mineral (Mg, Ca, Fe, Zn, K, P) and phytic acid content	Determination of Mg, Ca, Fe, Zn, K, P and phytic acid content	Material for association mapping and selection of interesting accessions (M) Data on seed mineral and phytic acid content (D)	Mirjana Herak Ćustić Marko Petek Igor Palčić Tomislav Karažija	from 7th to 12th month
DNA analysis	DNA extraction	DNA from 300 samples extracted and stored (D)	Klaudija Carović-Stanko Zlatko Liber Martina Grdiša Ivan Radosavljević	from 6th to 9th month
	Phaseolin type determination	Phaseolin type of 300 accessions determined (M)		from 9th to 11th month
	SSR markers	Amplification of DNA samples using 50 SSR markers (M); Fragment analysis (M); SSR maker data stored (D)		from 12th to 17th month
Construction of core collection	Selection of a subset representing 50% of whole collection	150 accessions selected for SNP analyses (D)	Klaudija Carović-Stanko Zlatko Liber Jerko Gunjača	from 18th to 24th month
DNA analysis	SNP markers	Whole genome variation characterization (M) Sup genotyping (D)	Contracted to a European Illumnia Certified Service provider	from 25th to 27th month
Data analyses	Genetic diversity and population structure evaluation; Association studies	Statistical data analyses (M); Interpretation of results (M); Results of the statistical analyses (D)	Klaudija Carović-Stanko Jerko Gunjača Zlatko Liber Marko Petek Boris Lazarević	from 22nd to 36th month

An economic method for the fluorescent labeling of PCR fragments

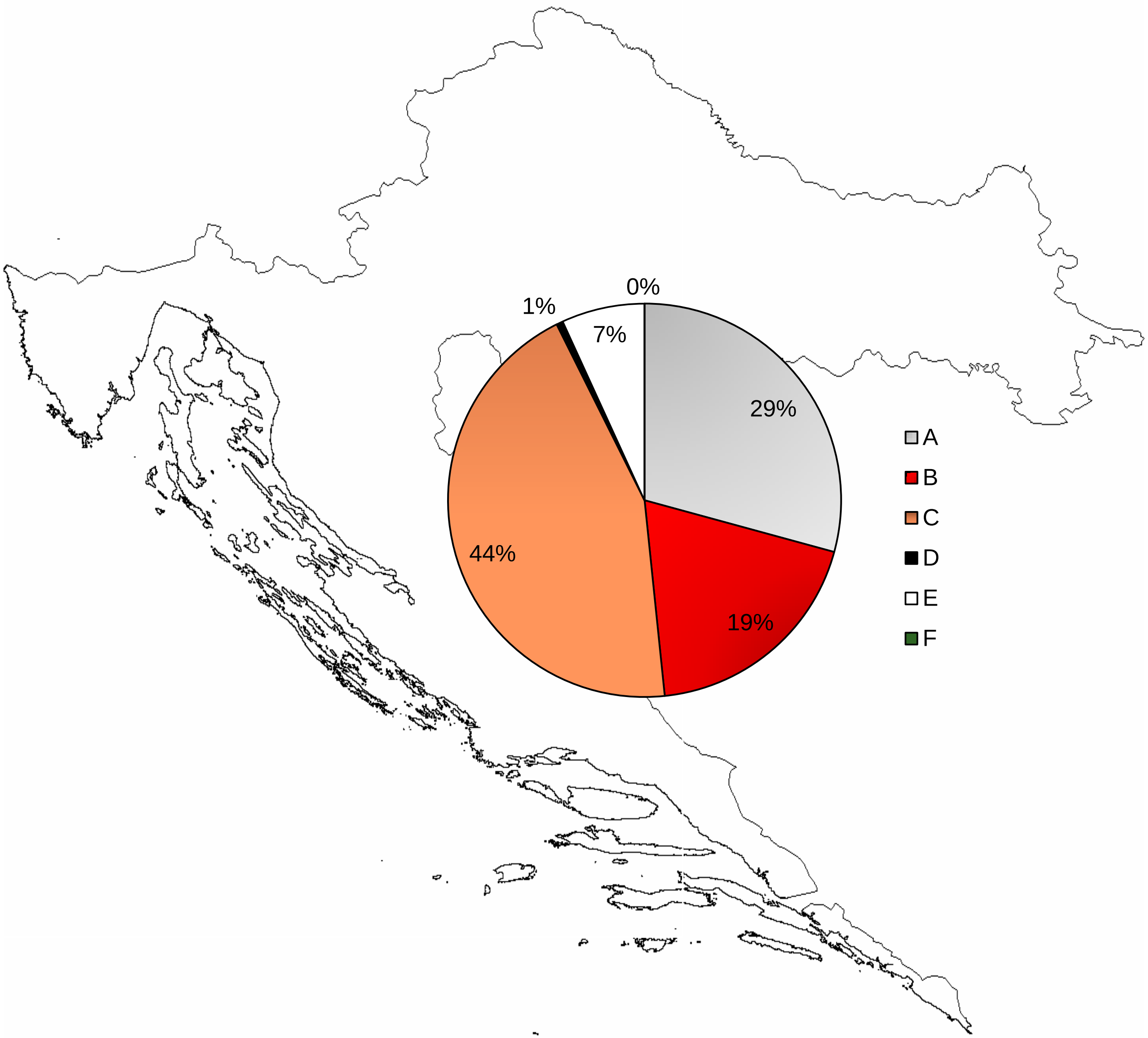
(Schuelke 2000)





Phaseolin type	Fragment size (bp)			
A	268	/	289	/
B	268	283	/	304
C	268	283	289*	304

Fazeolin tip hrvatskih tradicijskih kultivara graha



Dan noć

Trešnjevac

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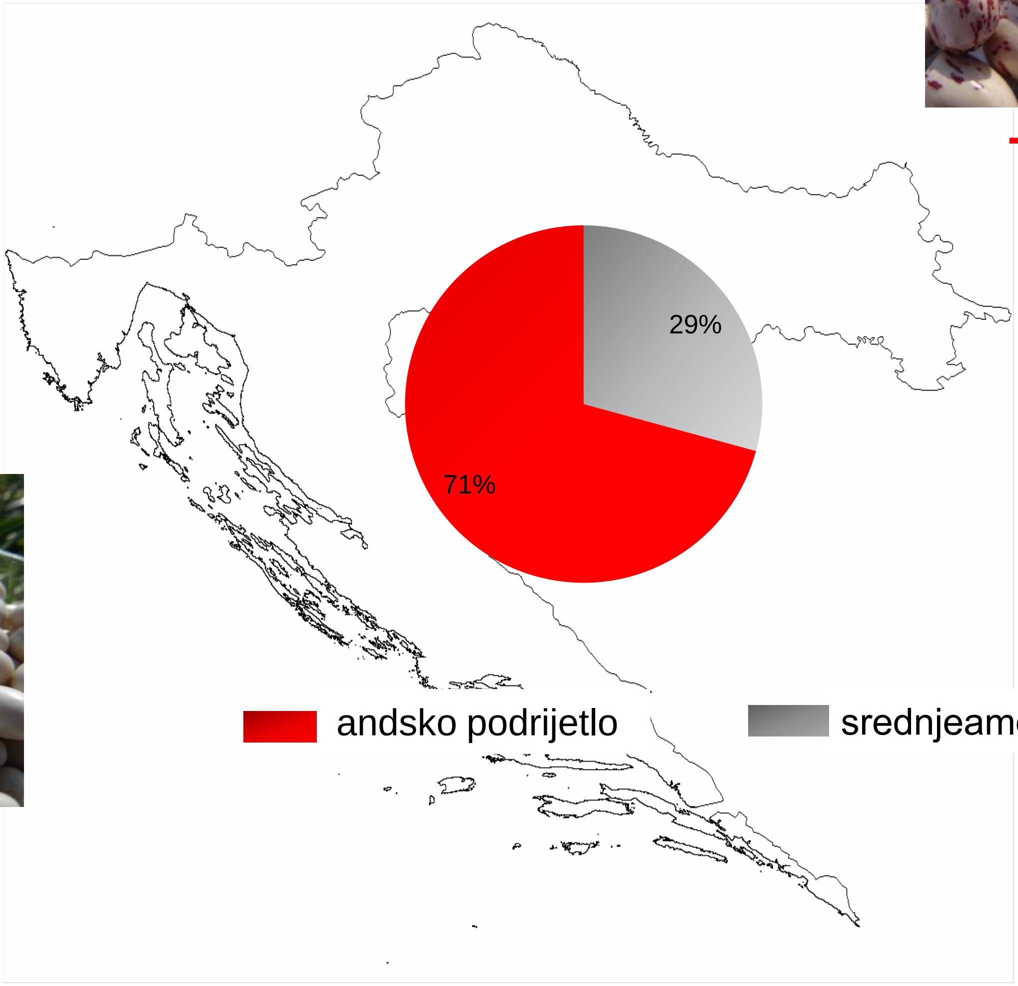
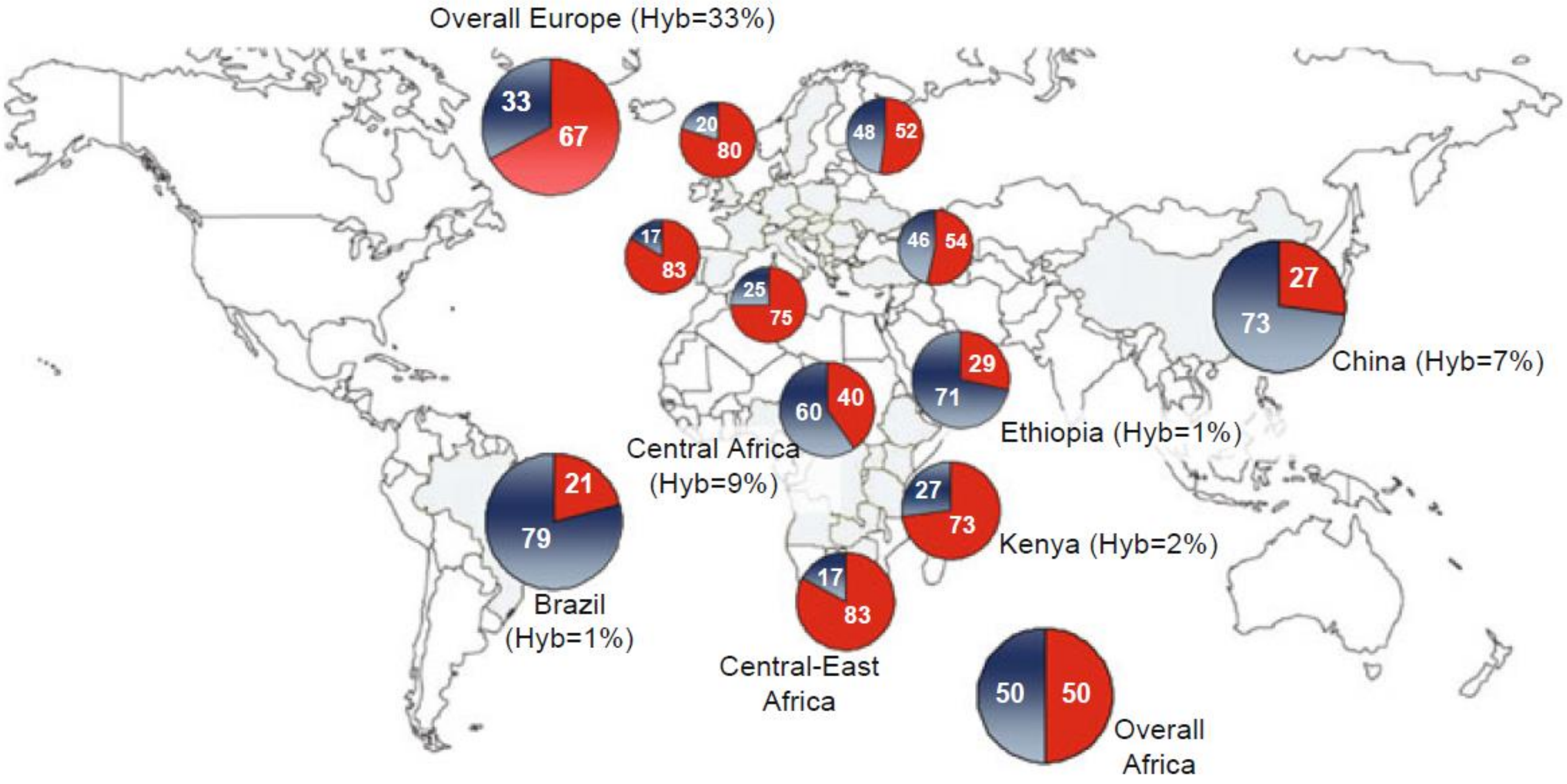
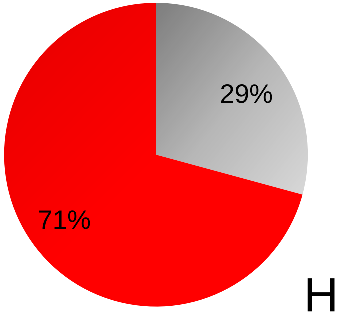


Podrijetlo hrvatskih tradicijskih kultivara graha





🟥 andsko podrijetlo

▨ srednjeameričko podrijetlo

Hrvatska

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